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(54) Therapeutic Uses of Eicosapentaenoic Acid

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ABSTRACT

Therapeutic Uses of Eicosapentaenoic Acid

The use of the omega-3 polyunsaturated fatty acid eicosapentaenoic acid or EPA, of high purity and preferably substantially free of any other polyunsaturated fatty acids, is disclosed for making up medical preparations or compositions for the treatment of cachexia and/or malignant tumours in mammals. Treatment with EPA is also useful for inhibiting lipolytic activity of biologically active lipolytic agents present in body fluids and the activity of the enzyme guanylinobenzotase, as well as for reducing abnormal CAMP levels in cells of adipose tissue in mammals, produced by lipolytic or fat mobilising substances.

THERAPEUTIC USES OF EICOSAPENTAENOIC ACID

This invention relates to the omega-3 poly-unsaturated fatty acid 5,8,11,14,17-eicosapentaenoic acid (herein referred to shortly as EPA, which term is to be understood as including also physiologically functional derivatives thereof, e.g. salts or esters), and to use in medicine of this compound for providing an active therapeutic agent. EPA is known to occur naturally as one of the several fatty acid constituents of marine oils, commonly called "fish oil".

BACKGROUND

The present invention has arisen out of experimental studies investigating a newly-identified biologically active substance having a high lipolytic activity which seems to be specifically associated with a range of malignant tumours, especially cachexia-inducing tumours, in animals and in humans. During the course of these studies, EPA was found to act as an effective antagonist or inhibitor of this so-called lipolytic factor; it was also found to be effective in depressing an abnormal elevated level of cyclic adenylic acid (cAMP) produced in adipose tissue cells (adipocytes) by this so-called lipolytic factor or by other known lipolytically active substances, as evidenced by in vitro experiments conducted using mouse adipose tissue cell preparations.

SUMMARY OF THE INVENTION

The present invention is based on the above findings and also on the further finding that administration of EPA on its own especially using material of high purity is effective in vivo in suppressing the symptoms of cachexia, particularly cancer cachexia, and/or in inhibiting or reducing tumour growth whereby it can

provide a useful active therapeutic agent for treatment of these conditions. These effects, it is believed, are related at least in part to in vivo activity peculiar to EPA in inhibiting the above-mentioned newly-identified lipolytic factor and in reducing abnormal elevated cAMP levels in adipose tissue cells produced by this or by other lipolytic agents. They may also be related to inhibition of proteolytic activity which has been noted in respect of skeletal muscle in some instances of tumour growth. It is, in fact, now thought that the anticachectic effect and anti-tumour effect are probably linked in that the former prevents or reduces the production of lipolytic metabolites required by the tumours concerned and this in turn results in an increased rate of tumour cell death.

It has also been found that EPA can have an inhibitory effect on guanidinobenzoatase which, it is believed, may promote an effect of EPA in reducing invasive and metastatic activities of malignant tumour cells.

Thus, according to one aspect of the present invention, 5,8,11,14,17-eicosapentaenoic acid or a physiologically functional derivative thereof, e.g. a salt or ester, herein collectively designated EPA and preferably of at least 75% purity, is used to make a medical preparation or medicament for the treatment of cachexia in mammals.

Also, according to the invention, EPA as specified above and of at least 75% purity is used to make a medical preparation or medicament for the treatment of malignant tumours in mammals.

Also, according to the invention, EPA as specified above is used to make a medical preparation or medicament

for therapeutic treatment to reduce abnormal cAMP levels in cells of adipose tissue in mammals produced by a lipolytic or fat mobilising substance present in body fluids or the circulatory system.

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The invention also comprises the use of EPA as specified above for the manufacture of a medical preparation or medicament for therapeutic treatment to inhibit lipolytic activity of biologically active lipolytic agents present in body fluids in mammals and/or activity of the enzyme guanidinobenzoate associated with tumour cells and cells capable of migration in mammals.

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The invention also comprises the use of EPA as specified for therapeutic treatment as herein disclosed.

In carrying out the invention, in general an effective anti-cachectic and/or antitumour amount of the EPA, or an effective amount of EPA for reducing an abnormal elevated level of cAMP in adipose tissue cells produced by a lipolytic or fat mobilising substance present in the body fluids or circulatory system of a mammal to be treated, will be made up as a pharmaceutical formulation ready for administration in any suitable manner, for example orally, parenterally (including subcutaneously, intramuscularly and intravenously), or topically. Such formulations may be presented in unit dosage form and may comprise a pharmaceutical composition, prepared by any of the methods well known in the art of pharmacy, in which the active EPA component is in intimate association or admixture with at least one other ingredient providing a compatible pharmaceutically acceptable carrier, diluent or excipient. Alternatively, such formulations may comprise a protective envelope of compatible or relatively inert pharmaceutically acceptable material within which is contained the active

EPA component in the form of concentrated or pure EPA without association or admixture with any other ingredients.

5 Formulations of the present invention suitable for oral administration may be presented as discrete units such as capsules, cachets, tablets or lozenges, each containing a predetermined amount of the active EPA component, with capsules being a preferred type of
10 formulation for providing the most effective means of oral delivery. For parenteral administration the formulations may comprise sterile liquid preparations of a predetermined amount of the active EPA component contained in sealed ampoules ready for use.

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Suitable dosage levels of the EPA, at least when used as an anticachectic or antitumour therapeutic agent, will generally be in excess of 100-1000mg per kilogram of body weight.

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In a further alternative definition the invention, from one aspect, may also be expressed as providing a composition for inhibiting lipolytic activity of biologically active lipolytic agents present in body
25 fluids in mammals and/or for inhibiting the enzyme guandibenzoatase associated with tumour cells and with cells capable of migration in mammals, characterised in that the composition comprises an effective inhibiting amount of EPA (as herein defined) together with a
30 compatible pharmaceutically acceptable material or carrier.

Preferably, especially for antitumour applications but also for anticachectic applications, the EPA used for
35 making the medical preparations, medicaments or compositions in accordance with the invention should be of at least about 80% purity and should contain no more

than minimal or pharmaceutically insignificant amounts of any other polyunsaturated fatty acids. A purity of about or more than 90% is recommended with the highest commercially available grade (about 95% purity), which is substantially free of any other polyunsaturated fatty acids, being the most preferred material.

Although EPA of high purity readily oxidises and is an inherently unstable compound under normal ambient conditions in the presence of air such that it usually requires to be stored out of sunlight at a low temperature under an atmosphere of nitrogen, difficulties in handling can be minimized by observing precautionary measures well known in the art. It will normally be protected from contact with air and sunlight in the pharmaceutical formulations into which it is made up for therapeutic use and such formulations may be kept stored at low temperatures until required for use.

By way of further background explanation and description of the invention, illustrative examples are hereinafter presented of investigations made and results obtained in the development of the invention, from which the skilled person in the art will more readily be able to appreciate the nature thereof and to put the invention into practical effect.

BRIEF DESCRIPTION OF THE DRAWINGS

In the accompanying drawings,

FIGURES 1 and 2 are diagrams showing the effect of EPA on lipolytic activity associated with MAC16 tumour extracts;

FIGURE 3 is a diagram showing the effect of EPA on proteolytic activity associated with MAC16 tumour extracts;

FIGURE 4 is a bar chart diagram showing the effect of EPA on the activity of several different lipolytic agents;

FIGURE 5 is a similar bar chart diagram showing the effect of EPA on the level of cAMP in fat cells or adipocytes during incubation with the different lipolytic agents referred to in FIGURE 4;

FIGURE 6 is a diagram showing the inhibition effect of EPA on guanidinobenzoate;

FIGURE 7 is a diagram showing the effect of oral dosing with EPA on the growth of MAC16 adenocarcinoma in female NMRI mice;

FIGURE 8 is a similar diagram showing the effect of oral dosing with EPA on body weight of female NMRI mice bearing the MAC16 tumour;

FIGURES 9 and 10 are further diagrams similar to FIGURES 7 and 8 showing additional comparative results; and

FIGURES 11A and 11B and FIGURES 12A and 12B are also further diagrams similar to FIGURES 7 and 8 illustrating the results of additional in vitro experiments.

MORE DETAILED DESCRIPTION

In some preliminary experiments using mice, evidence was first obtained suggesting that EPA could inhibit the growth in vivo of at least two different tumours, designated MAC16 and MAC13 respectively, belonging to an established series (MAC) of chemically induced, transplantable colon adenocarcinomas (see for example Cowen et al (1980), JNCI, 64, 675-681), and could reduce the weight loss or cachexia associated with the MAC16 tumour. The MAC16 tumour is a cachexia-inducing

tumour associated with high levels of lipolytic activity and also with proteolytic activity, and it is generally resistant to antitumour agents that interfere with cell production; the MAC13 is (at least in mice) a non-cachexia inducing colon adenocarcinoma (albeit extracts thereof do show a certain level of lipolytic activity). Subsequent studies in vivo with the MAC16 tumour further indicated that the reduction in weight loss and inhibition of the apparently related tumour-associated lipolytic activity referred to above, as well as the tumour growth inhibition, was a dose related effect. However, the anticachectic effect of EPA has been found, somewhat surprisingly, to exceed the antitumour effect.

During the course of continuing investigations of the previously mentioned newly identified so-called lipolytic factor, derived from the above-mentioned tumours, a series of in vitro experiments was conducted to screen a range of various compounds, including EPA, for possible activity as inhibitors or antagonists to the lipolytic factor. In general, in these experiments the compounds to be tested were added to extracts from MAC16 tumours and incubated with freshly prepared adipocytes from mouse epididymal adipose tissue for 2 hrs. The lipolytic activity, or reduction thereof, was then determined by measuring the glycerol release using an enzyme assay that results in a production of NAD (nicotinamide adenine dinucleotide) from the reduced form NADH, the amount of NAD corresponding to the amount of glycerol present. The NAD was measured spectrophotometrically as a decrease in absorption at 340nm.

More specific details of the experimental procedures in these initial inhibition studies are summarised below:

1. Preparation of extracts from MAC16 tumours.
MAC16 tumours from NMRI mice that had lost up to one

third of their original body weight, were homogenised in Krebs-Ringer buffer at a concentration of 0.2g/ml. The homogenate was then centrifuged and the supernatant used for inhibition studies.

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2. Preparation of adipocytes

Fat pads were removed from 2 mice for the assay of each batch of 10 samples. 1 ml of collagenase solution in Krebs buffer (2 mg/ml) was added to the fat pads from 1 mouse which were then finely chopped prior to incubation for 2 hr at 37°C. After 2 hr the adipocytes were pooled, washed three times in Krebs buffer, and then counted to obtain a concentration of $1.5 - 2.0 \times 10^5$ adipocytes per ml.

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3. The experiment was set up as follows.

100µl tumour extract + 1 ml fat cells
Compound to be screened + 1 ml fat cells
100µl tumour and compound + 1 ml fat cells

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Each compound was tested at increasing concentrations and all samples were prepared and processed in duplicate.

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The samples were gassed for 2 min with 95% O₂, 5% CO₂ mixture, mixed and incubated for 2 hr at 37°C. After 2 hr, 0.5 ml from each sample was then assayed for glycerol content.

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These experiments confirmed that EPA at a sufficient dosage has a strong inhibitory effect on the lipolytic activity of the lipolytic factor in the tumour extracts, as illustrated for example by FIGURE 1 of the accompanying drawings. This diagram shows the results obtained using EPA in one set of the above experiments and it also clearly indicates the dose dependence nature of the effect. In FIGURE 2, the same results are

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presented as a Dixon plot. Lipolytic activity is expressed as μmol glycerol released from the murine epididymal adipocytes per mg protein per hr. Results are expressed as mean \pm S.E.M and the number of experiments performed was 3 to 4.

Similar tests on a range of compounds, including other related polyunsaturated fatty acids, failed to indicate any significant inhibition of this lipolytic factor in the tumour extracts by gamma-linolenic acid, octadecatetraenoic acid, trans-3-hexenoic acid, trans 2-hexenoic acid, cis-3-hexen-1-ol, 3-octenoic acid, linoleic acid, eicosatrienoic acid, arachidonic acid, palmitoleic acid, nicotinic acid, adenosine or inosine. As will hereinafter appear, docosahexaenoic acid also appears not to act as an inhibitor of the activity of this tumour lipid mobilising agent or lipolytic factor.

In addition, in similar experiments wherein extracts of MAC16 tumours were incubated with mouse diaphragm, EPA was found to inhibit the proteolytic activity, as shown for example in FIGURE 3 in which the results of typical experiments are again presented as a Dixon plot. In this diagram, proteolytic activity of the tumour extracts is expressed in terms of nmoles total amino acid released per gram diaphragm per mg protein per 2hr and are corrected for spontaneous amino acid release. Results are expressed as mean \pm S.E.M and the number of experiments performed was 3 to 4.

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In a further series of similar experiments, EPA was found to act as an effective inhibitor not only of the lipolytic factor from the tumour extracts referred to above, but also of some other substances that are known to function as biologically active lipolytic agents. These included ACTH (the lipase activating adrenocorticotrophic hormone) and Salbutamol, the effects being

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illustrated in FIGURE 4 of the accompanying drawings which is a bar chart diagram showing the relative levels of lipolytic activity detected under comparable conditions for the various test samples enumerated.

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In the course of these latter experiments, tests were also carried out to measure (by known techniques) the level of cyclic adenylic acid (cAMP) in the fat cells or adipocytes during the period of incubation with the various lipolytic agents, both in the presence of and without the presence of EPA. The results, after a 10 minute incubation period, are presented in the bar chart diagram of FIGURE 5 of the accompanying drawings which should be compared with FIGURE 4. The results in FIGURE 5 are expressed as the Mean \pm S.E.M of 3 to 4 experiments. It will be seen that each of the three specific lipolytic agents tested, when present on its own and lipolytically most active, gives rise to an abnormal elevated level of cAMP in the cells, but this elevated level is depressed in the presence of EPA. This suggests that normally, like many hormones, these lipolytic agents bind to specific receptor sites on the membrane of the fat cells or adipocytes concerned and act to bring about a modification of the intracellular level of cAMP which is known to act as a so-called secondary hormone that regulates the activity of enzyme systems within the cell. The indications are that the EPA interferes with and inhibits this effect, acting specifically somewhere in the adenylate cyclase cascade to inhibit the production of cAMP in response to lipolytic stimuli, perhaps by itself binding to and blocking the receptor sites involved. Again, this property has been found to be unique to EPA and is not shown by other polyunsaturated fatty acids.

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In addition, as illustrated by the Dixon plot of results shown in FIGURE 6, EPA has been found to be a

highly effective inhibitor of the enzyme guanidino-benzoatase which is a trypsin-like enzyme associated with tumour cells and cells capable of migration and which, it has been suggested, may play a role in tumour metastasis.

5 In deriving the results shown in FIGURE 6, enzyme activity was determined by the release of the fluorogenic product methyl umbelliferone from 4-methylumbelliferyl-p-guanidinobenzoate by 1 μ l of NMRI mouse serum. The inhibitor was added to the enzyme together with the
10 substrate and the decrease of fluorescence at 446nm was determined. In this property EPA is again also unique in that it appears to be the only fatty acid capable of effectively inhibiting guanidinobenzoatase.

15 In continuing in vivo experiments, it was found, surprisingly, that EPA of high purity administered on its own is significantly more effective as a therapeutic agent, particularly as an antitumour agent, than had been anticipated and also it appears to have a very low
20 toxicity. Tests were first carried out to compare the effects of oral dosing of 95% pure EPA in the form of the triglyceride ester (obtained from Peninsula Laboratories, Merseyside, United Kingdom) with the effects of similar dosing of the omega-6 polyunsaturated fatty acid linoleic
25 acid using mice bearing the MAC16 colon adenocarcinoma. The dose level was 5g kg⁻¹, and no toxicity or other adverse effects were noted. The results, as illustrated in FIGURE 7, showed EPA at this dose level to produce an extensive growth delay. In obtaining these results, the
30 mice (20g weight) were dosed orally with either 100 μ l (pure) EPA/day/mouse (A), 100 μ l linoleic acid/day/mouse (B) or (as a control) 100 μ l 0.9% saline/day/mouse (C). The experiment was initiated 14 days after tumour transplantation when the tumours became palpable (average
35 initial tumour volume = 176 \pm 23 mm³). Tumour volumes were measured daily and recorded as a percentage of the tumour volume prior to oral dosing. Results are expressed as

the mean \pm S.E.M.

The effect of these fatty acids on host body weight is shown by the results illustrated in FIGURE 8. Again
5 mice (20g) were dosed orally with either 100 μ l (pure) EPA/day/mouse (A), 100 μ l linoleic acid/day/mouse (B) or 100 μ l 0.9% saline/day/mouse (C). Treatment was initiated 14 days after tumour transplantation when weight loss became apparent (average weight loss 5%). In this case,
10 body weights were measured daily and recorded as a percentage of the body weight prior to oral dosing, the results again being expressed as the mean \pm S.E.M. There was no significant difference in food and water intake. As will be seen, the results show EPA also effectively
15 blocked the cachectic effect of the tumour whereas linoleic acid at an equivalent dose level had an enhancing effect and increased weight loss.

In further similar experiments, of which the results
20 are shown in FIGURE 9 and 10, the effect of dosing with pure EPA was also compared with the effect of linoleic acid and with that of docosahexaenoic acid (purchased from Sigma Chemical Co., Poole, U.K.), herein referred to shortly as DHA.

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FIGURES 9 and 10 shows the results of experiments in which again mice (20g) were used and dosed orally with either 100 μ l (pure) EPA/day/mouse (A), 100 μ l linoleic acid/day/mouse (B), 100 μ l 0.9% saline/day/mouse (C) or
30 100 μ l DHA/day/mouse (D).

Again, the experiment of which the results are shown in FIGURE 9 was initiated 14 days after tumour transplantation when the tumours became palpable (average
35 initial tumour volume = $96 \pm 12 \text{ mm}^3$). Tumour volumes were measured daily and recorded as a percentage of the tumour volume prior to oral dosing. Results are expressed as

the mean \pm S.E.M.

The effect on host body weight from the treatment relating to FIGURE 9 is shown in FIGURE 10. The treatment was also initiated 14 days after tumour transplantation when weight loss became apparent (average weight loss 7%). Body weights were measured daily and recorded as a percentage of the body weight prior to oral dosing. Results are expressed as the mean \pm S.E.M. There was no significant difference in food and water intake.

The results presented in these FIGURES 9 and 10 demonstrate clearly that only EPA possesses antitumour and anticachectic activity. In contrast, the related omega-3 polyunsaturated fatty acid DHA, which is also a major constituent of fish oil (18.7%) and which differs from EPA only in the presence of two extra carbon atoms and another double bond, was totally devoid of any antitumour or anticachectic activity, when administered orally at the same dose as EPA (5g kg^{-1}). Moreover, while EPA was non-toxic at this dose level, DHA showed marked signs of toxicity, as evidenced by an increased weight loss compared with the control, and the experiments had to be terminated after only a few oral doses. It has also been ascertained that administration of EPA significantly reduces the level of arachidonic acid (ARA) which appears in the blood plasma of animals bearing the MAC16 tumour. A similar effect has also been found with administration of DHA, but since only EPA exerts antitumour and anticachectic activity against the MAC16 tumour, this indicates that reductions in tumour ARA, leading perhaps to an inhibition of prostaglandin synthesis, at least in themselves are not of prime importance for either activity.

Apart from DHA, it is also significant that the use

of gamma-linolenic acid (GLA) was found to be ineffective in showing any antitumour or anticachectic activity when tested in similar experiments with mice bearing the MAC16 tumour.

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The effect of using EPA mixed with other polyunsaturated fatty acids was further investigated in another set of experiments of which the results are illustrated in FIGURES 11A and 11B and FIGURES 12A and 12B. In this case EPA of 80% purity (from Scotia Pharmaceuticals Ltd., Guildford, United Kingdom) was used, and the experiments were performed on female NMR mice (20g weight average) again bearing the MAC16 colon adenocarcinoma and commencing to exhibit weight loss. Administration of the fatty acids was carried out by gavage and the general procedure was similar to that of the experiments previously described.

FIGURES 11A and 11B respectively show the effect on tumour growth and on host body weight of dosing with 80% pure EPA (E) at a dosage of 2.5g Kg^{-1} (75 $\mu\text{l/day}$), with 80% EPA diluted with other fatty acids to 57% purity and administered (75 $\mu\text{l/day}$) at the same concentration of EPA as the 80% pure material (F), and with water (C) as a control. The 57% EPA was obtained by diluting with a mixture of docosahexaenoic acid (DNA), palmitoleic acid, oleic acid, stearic acid and palmitic acid. The materials were sealed under nitrogen and were free of antioxidants.

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It is seen that the growth of the MAC16 tumour in the animals treated with the 80% EPA (E) had two distinct phases (FIG. 11A), namely a stationary phase where tumour growth was almost completely suppressed and an exponential phase where tumour growth continued at a rate similar to that in the non-treated control animals (C). This antitumour activity appeared to be primarily due to

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an increase in the tumour cell loss factor during the stationary phase. When, however, the 80% EPA was diluted with the other fatty acids it was found to be more toxic and without apparent antitumour activity (F). FIGURE 11B shows clearly the anticachectic activity of the pure EPA. This anticachectic activity was in fact retained to some extent when using the 57% pure material, but the higher toxicity of this impure material presented a problem for continuous treatment.

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FIGURES 12A and 12B illustrate the results obtained in further experiments similar to those described in relation to FIGURES 11A and 11B except that in addition to dosing with 80% pure EPA (2.0g Kg^{-1}) (curve E), some of the animals were dosed (1.9g Kg^{-1}) with linoleic acid (76.7% with 21.6% oleic acid) alone (curve G) and others were dosed with EPA (80%) in combination with linoleic acid (2.5g Kg^{-1}) (curve H). As shown in FIG. 12A, the linoleic acid alone (G) was again found to increase weight loss (as curve B in FIGS. 8 and 10), although the mixture of EPA and linoleic acid (H) was still effective in preventing or reducing weight loss. On the other hand, in relation to tumour growth (FIG. 12B), whilst linoleic acid alone (G) appeared to stimulate growth in contrast to the reduction or suppression obtained with 80% EPA alone (E), the mixture of EPA and linoleic acid (H) was virtually without significant effect and growth was similar to that of the untreated control (C). Again, there was no significant difference in food or water intake between the different groups of animals in these experiments.

These results further confirm that in respect of such antitumour and anticachectic effects as have been found, not only is EPA of high purity, at least 75-80% pure and substantially free of any other polyunsaturated fatty acids (especially DHA), even more efficacious,

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particularly in respect of antitumour activity but also in some cases in respect of anticachectic activity, and is less toxic than anticipated. Overall, EPA appears to have special properties not shared by the other poly-
5 unsaturated fatty acids.

Thus, the inhibiting or antagonistic effects found for EPA against lipolytic agents that are known to be physiologically active and often associated with a
10 condition of cachexia and also with tumour growth, such as the newly-identified lipolytic factor produced by MAC16 adenocarcinoma tumours, have demonstrated an unexpected high potential for high purity EPA as a valuable therapeutic agent, especially in the treatment
15 of cachexia and/or tumours in mammals, particularly as such newly-identified lipolytic factor is now known to be associated with many other tumours including, it is believed, many if not most human tumours.

20 Although there have been some previous reports indicating an antitumour activity of compositions containing certain polyunsaturated fatty acids including EPA, hitherto it has generally been believed that it has been the total fatty acid content that has been
25 important, especially the total omega-3 polyunsaturated fatty acids including docosahexaenoic acid in the case of fish oils, and there has been no clear recognition of the specific nature of the effect of EPA, its anticachectic activity per se, and the desirability or importance of
30 using this material for therapeutic purposes in a high state of purity without other polyunsaturated fatty acids. In particular, in many cases results obtained in vitro with impure preparations and in the presence of other polyunsaturated fatty acids have not been
35 reproducible in vivo especially with tumours that tend to be generally resistant to antiproliferative agents.

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THE EMBODIMENTS OF THE INVENTION IN WHICH AN EXCLUSIVE PROPERTY OR PRIVILEGE IS CLAIMED ARE DEFINED AS FOLLOWS:

1. Use of 5,8,11,14,17-eicosapentaenoic acid or a physiologically functional derivative thereof (herein collectively designated EPA) for the manufacture of a medical preparation or medicament for the treatment of cachexia in mammals.
2. Use of EPA as claimed in Claim 1 wherein an effective anti-cachectic amount of the EPA of at least 75% purity is incorporated in the medical preparation or medicament which is made up as a pharmaceutical formulation in unit dosage form.
3. Use of 5,8,11,14,17-eicosapentaenoic acid or a physiologically functional derivative thereof (herein collectively designated EPA) for the manufacture of a medical preparation or medicament for therapeutic treatment to reduce abnormal cAMP levels in cells of adipose tissue in mammals produced by lipolytic or fat mobilising substances in body fluids thereof.
4. Use of EPA as claimed in Claim 3 wherein an amount of the EPA of at least 75% purity that is effective to reduce said abnormal elevated level of cAMP in adipose tissue cells of the mammal to be treated is incorporated in the medical preparation or medicament which is made up as a pharmaceutical formulation in unit dosage form.
5. Use of 5,8,11,14,17-eicosapentaenoic acid or a physiologically functional derivative thereof (herein collectively designated EPA) for the manufacture of a medical preparation or medicament for therapeutic treatment to inhibit abnormal lipolytic activity in mammals produced by biologically active lipolytic agents present in body fluids thereof.

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6. Use of EPA as claimed in Claim 5 wherein an effective lipolytic agent inhibiting amount of the EPA of at least 75% purity is incorporated in the medical preparation or medicament which is made up as a pharmaceutical formulation in unit dosage form.
7. Use of 5,8,11,14,17-eicosapentaenoic acid or a physiologically functional derivative thereof (herein collectively designated EPA) for the manufacture of a medical preparation or medicament for therapeutic treatment of mammals to inhibit tumour metastasis.
8. Use of EPA as claimed in Claim 7 wherein an effective guanidinobenzoatase inhibiting amount of the EPA of at least 75% purity is incorporated in the medical preparation or medicament which is made up as a pharmaceutical formulation in unit dosage form.
9. Use of EPA as claimed in any one of Claims 1 to 8, wherein the EPA is of at least 80% purity.
10. Use of EPA as claimed in any one of Claims 1 to 8, wherein the EPA is of at least 90% purity.
11. Use of EPA as claimed in any one of Claims 1 to 8 wherein the EPA is about 95% pure.
12. Use of EPA as claimed in any one of Claims 1 to 8 wherein the EPA is substantially free of any other polyunsaturated fatty acids.
13. A method of inhibiting the enzyme guanidinobenzoatase and tumour metastasis in mammals which comprises administering to a host in need of such inhibition an effective guanidinobenzoatase inhibiting amount of 5,8,11,14,17-eicosapentaenoic acid or a physiologically functional derivative thereof (herein

collectively designated EPA).

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14. A method of inhibiting biologically active lipolytic agents producing abnormal lipolytic activity in mammals which comprises administering to a host in need of such inhibition an effective lipolytic agent inhibiting amount of 5,8,11,14,17-eicosapentaenoic acid or a physiologically functional derivative thereof (herein collectively designated EPA).

15. Use of 5,8,11,14,17-eicosapentaenoic acid or a physiologically functional derivative thereof (herein collectively designated EPA) for the manufacture of a medical preparation or medicament for the treatment of malignant tumours in mammals, characterised in that said EPA is of at least 80% purity.

16. Use of EPA as claimed in Claim 15 wherein the EPA is of at least 90% purity.

17. Use of EPA as claimed in Claim 15 wherein the EPA is about 95% pure.

18. Use of 5,8,11,14,17-eicosapentaenoic acid or a physiologically functional derivative thereof (herein collectively designated EPA) for the manufacture of a medical preparation or medicament for the treatment of malignant tumours in mammals, characterised in that said EPA is substantially free of any other polyunsaturated fatty acids.

19. A pharmaceutical formulation comprising a medical preparation or medicament manufactured using EPA of high purity in accordance with any one of Claims 15 to 18.

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20. Use of 5,8,11,14,17-eicosapentaenoic acid or a physiologically functional derivative thereof (herein collectively designated EPA) in a medical preparation or medicament for the treatment of cachexia in mammals.

21. Use of EPA as claimed in Claim 20 wherein an effective anti-cachectic amount of the EPA of at least 75% purity is incorporated in the medical preparation or medicament which is made up as a pharmaceutical formulation in unit dosage form.

22. Use of 5,8,11,14,17-eicosapentaenoic acid or a physiologically functional derivative thereof (herein collectively designated EPA) in a medical preparation or medicament for therapeutic treatment to reduce abnormal cAMP levels in cells of adipose tissue in mammals produced by lipolytic or fat mobilising substances in body fluids thereof.

23. Use of EPA as claimed in Claim 3 wherein an amount of the EPA of at least 75% purity that is effective to reduce said abnormal elevated level of cAMP in adipose tissue cells of the mammal to be treated is incorporated in the medical preparation or medicament which is made up as a pharmaceutical formulation in unit dosage form.

24. Use of 5,8,11,14,17-eicosapentaenoic acid or a physiologically functional derivative thereof (herein collectively designated EPA) in a medical preparation or medicament for therapeutic treatment to inhibit abnormal lipolytic activity in mammals produced by biologically active lipolytic agents present in body fluids thereof.

25. Use of EPA as claimed in Claim 24 wherein an effective lipolytic agent inhibiting amount of the EPA of at least 75% purity is incorporated in the medical preparation or medicament which is made up as a pharmaceutical formulation in unit dosage form.
26. Use of 5,8,11,14,17-eicosapentaenoic acid or a physiologically functional derivative thereof (herein collectively designated EPA) in a medical preparation or medicament for therapeutic treatment of mammals to inhibit tumour metastasis.
27. Use of EPA as claimed in Claim 26 wherein an effective guanidinobenzoate inhibiting amount of the EPA of at least 75% purity is incorporated in the medical preparation or medicament which is made up as a pharmaceutical formulation in unit dosage form.
28. Use of EPA as claimed in any one of Claims 20, 21, 22, 23, 24, 25, 26, or 27, wherein the EPA is of at least 80% purity.
29. Use of EPA as claimed in any one of Claims 20, 21, 22, 23, 24, 25, 26 or 27, wherein the EPA is of at least 90% purity.
30. Use of EPA as claimed in any one of Claims 20, 21, 22, 23, 24, 25, 26 or 27, wherein the EPA is about 95% pure.
31. Use of EPA as claimed in any one of Claims 20, 21, 22, 23, 24, 25, 26 or 27, wherein the EPA is substantially free of any other polyunsaturated fatty acids.

32. Use of 5,8,11,14,17-eicosapentaenoic acid or a physiologically functional derivative thereof (herein collectively designated EPA) in a medical preparation or medicament for the treatment of malignant tumours in mammals, characterised in that said EPA is of at least 80% purity.

33. Use of EPA as claimed in Claim 32 wherein the EPA is of at least 90% purity.

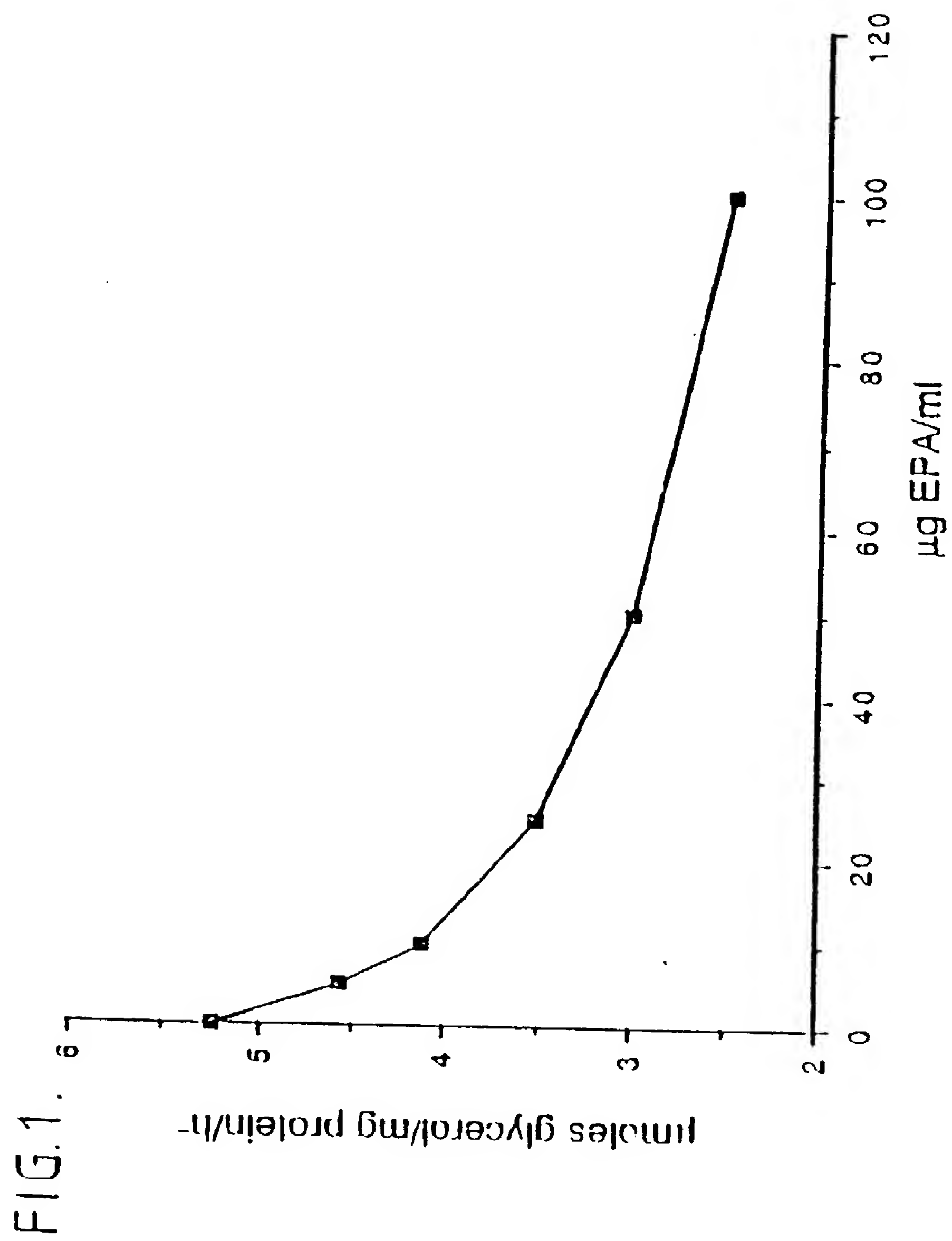
34. Use of EPA as claimed in Claim 32 wherein the EPA is about 95% pure.

35. Use of 5,8,11,14,17-eicosapentaenoic acid or a physiologically functional derivative thereof (herein collectively designated EPA) in a medical preparation or medicament for the treatment of malignant tumours in mammals, characterised in that said EPA is substantially free of any other polyunsaturated fatty acids.

36. Use of 5,8,11,14,17-eicosapentaenoic acid or a physiologically functional derivative thereof (herein collectively designated EPA) for the treatment of cachexia in mammals.

37. Use of 5,8,11,14,17-eicosapentaenoic acid or a physiologically functional derivative thereof (herein collectively designated EPA) for therapeutic treatment to reduce abnormal cAMP levels in cells of adipose tissue in mammals produced by lipolytic or fat mobilising substances in body fluids thereof.

38. Use of 5,8,11,14,17-eicosapentaenoic acid or a physiologically functional derivative thereof (herein collectively designated EPA) for therapeutic treatment to inhibit abnormal lipolytic activity in mammals produced by biologically active lipolytic agents present in body fluids thereof.
39. Use of 5,8,11,14,17-eicosapentaenoic acid or a physiologically functional derivative thereof (herein collectively designated EPA) for therapeutic treatment of mammals to inhibit tumour metastasis.
40. Use of 5,8,11,14,17-eicosapentaenoic acid or a physiologically functional derivative thereof (herein collectively designated EPA) for the treatment of malignant tumours in mammals, characterised in that said EPA is of at least 80% purity.
41. Use of EPA as claimed in Claim 40 wherein the EPA is of at least 90% purity.
42. Use of EPA as claimed in Claim 40 wherein the EPA is about 95% pure.
43. Use of 5,8,11,14,17-eicosapentaenoic acid or a physiologically functional derivative thereof (herein collectively designated EPA) for the treatment of malignant tumours in mammals, characterised in that said EPA is substantially free of any other polyunsaturated fatty acids.



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FIG. 2.

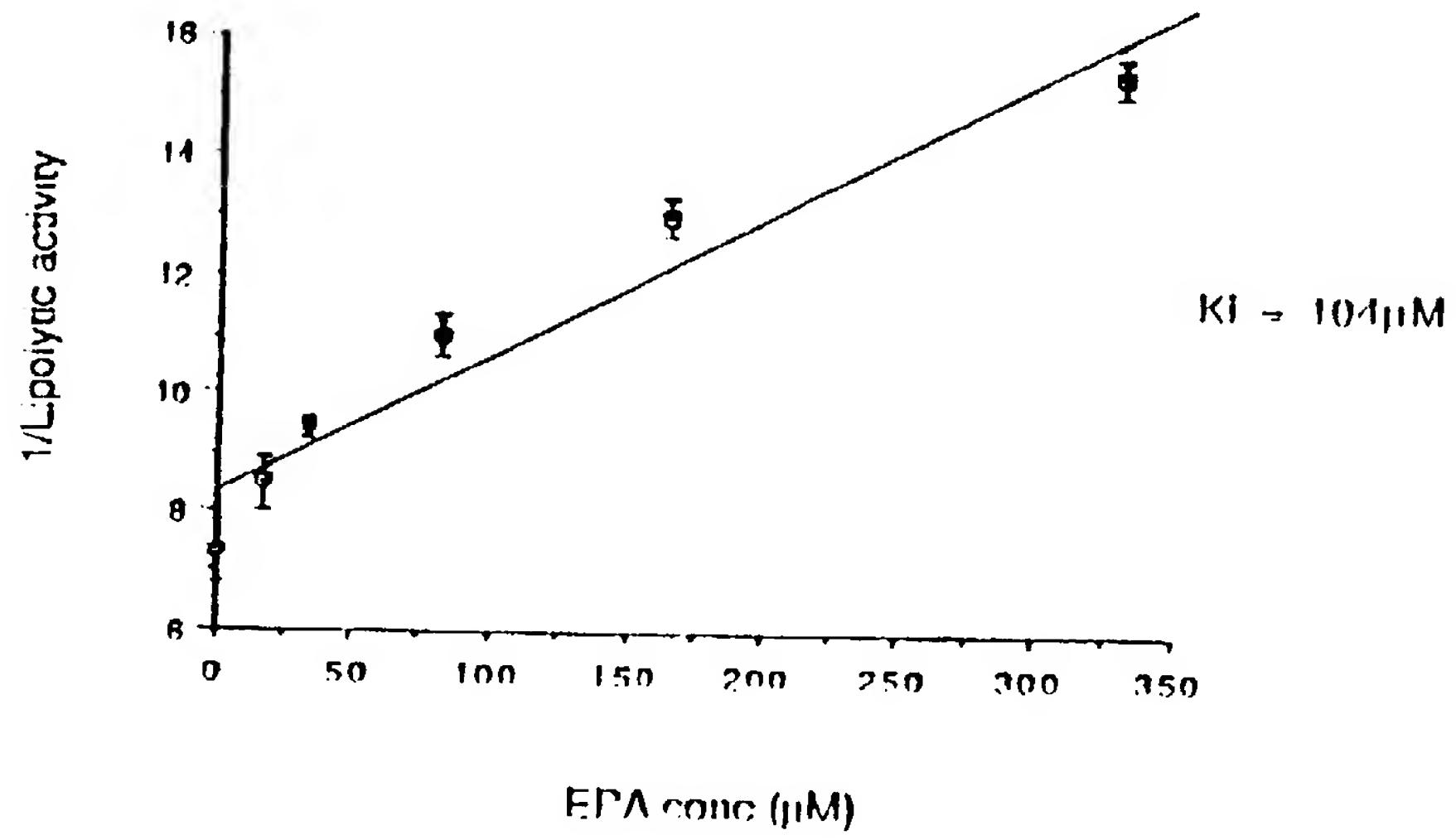
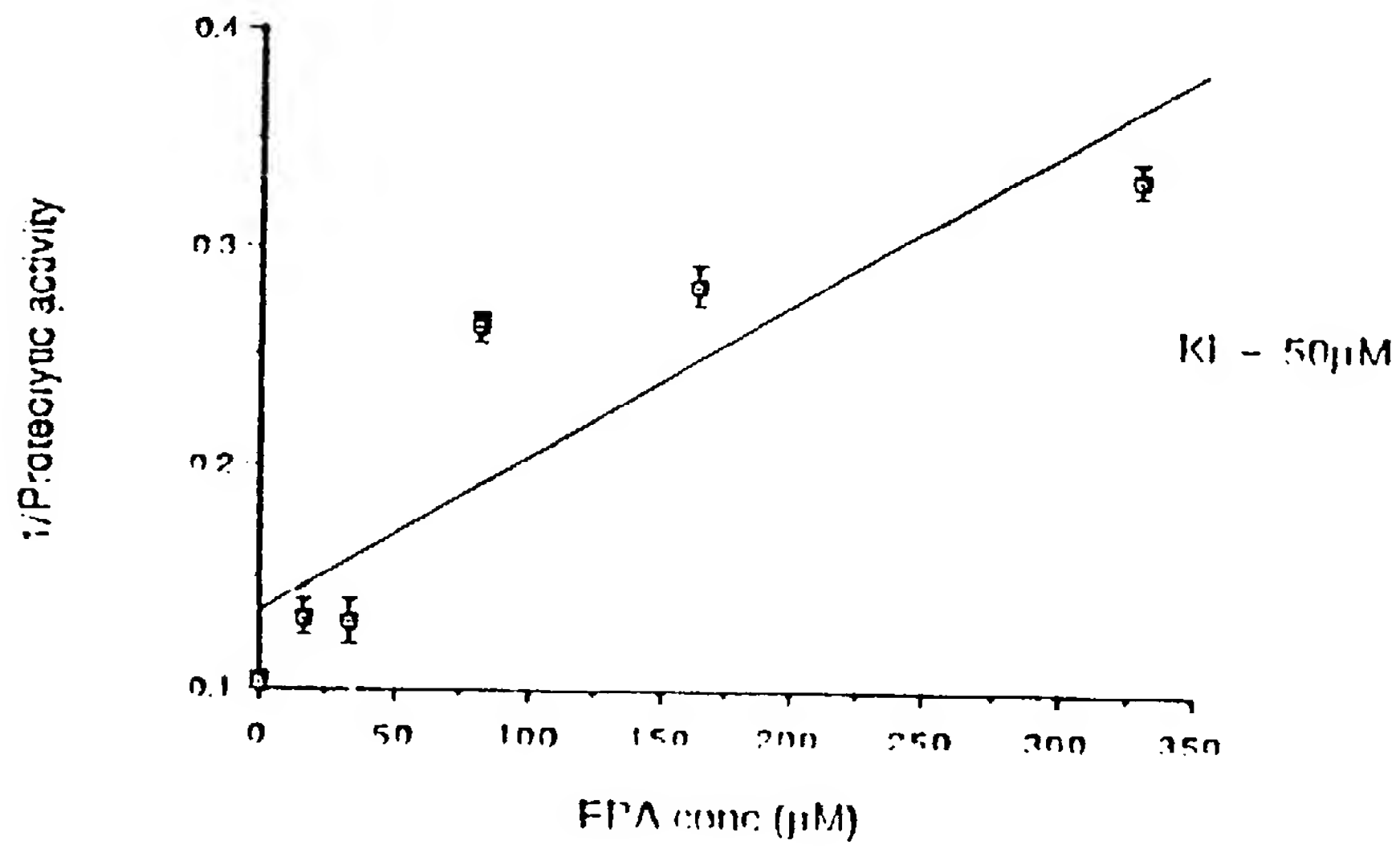


FIG. 3.

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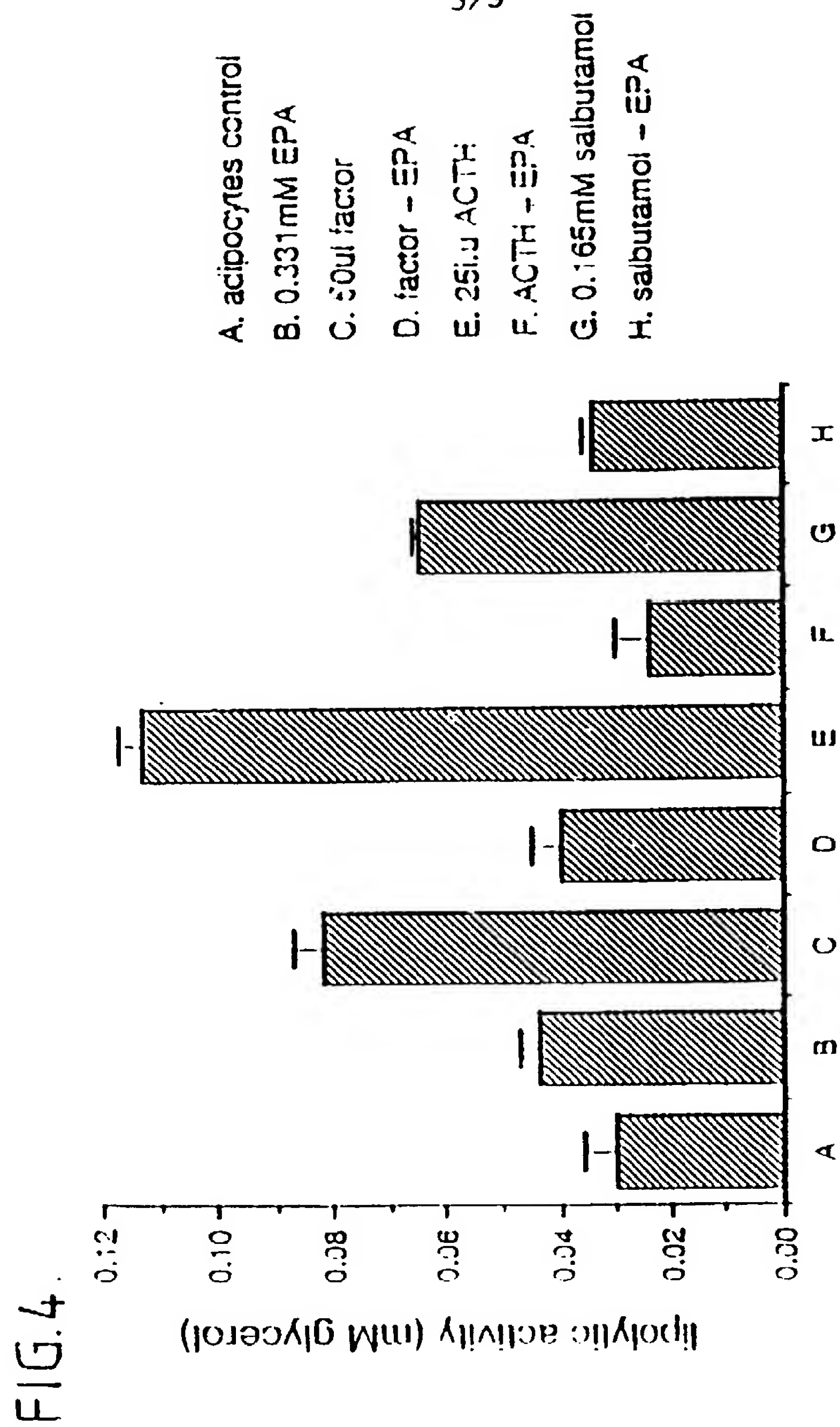
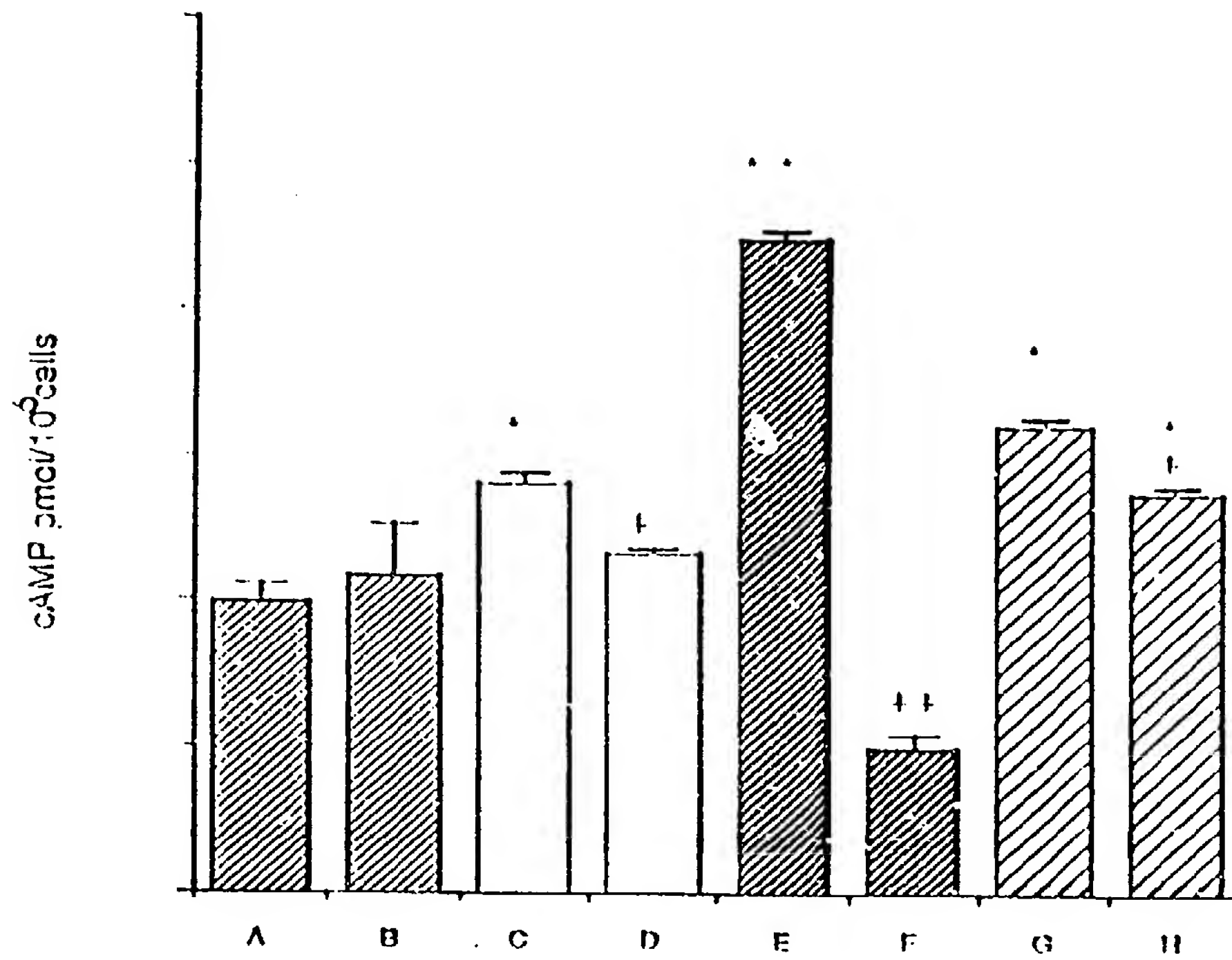
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FIG. 5.



- A. Control cells only
 B. 0.331mM EPA
 C. 50μl factor (0.082 μmoles glycerol released/10⁵ adipocytes/2hr)
 D. 50μl factor + 0.331mM EPA
 E. 25units ACTH
 F. 25 units ACTH + 0.331mM EPA
 G. 0.165mM Salbutamol
 H. 0.165mM Salbutamol + 0.331mM EPA

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FIG. 6.

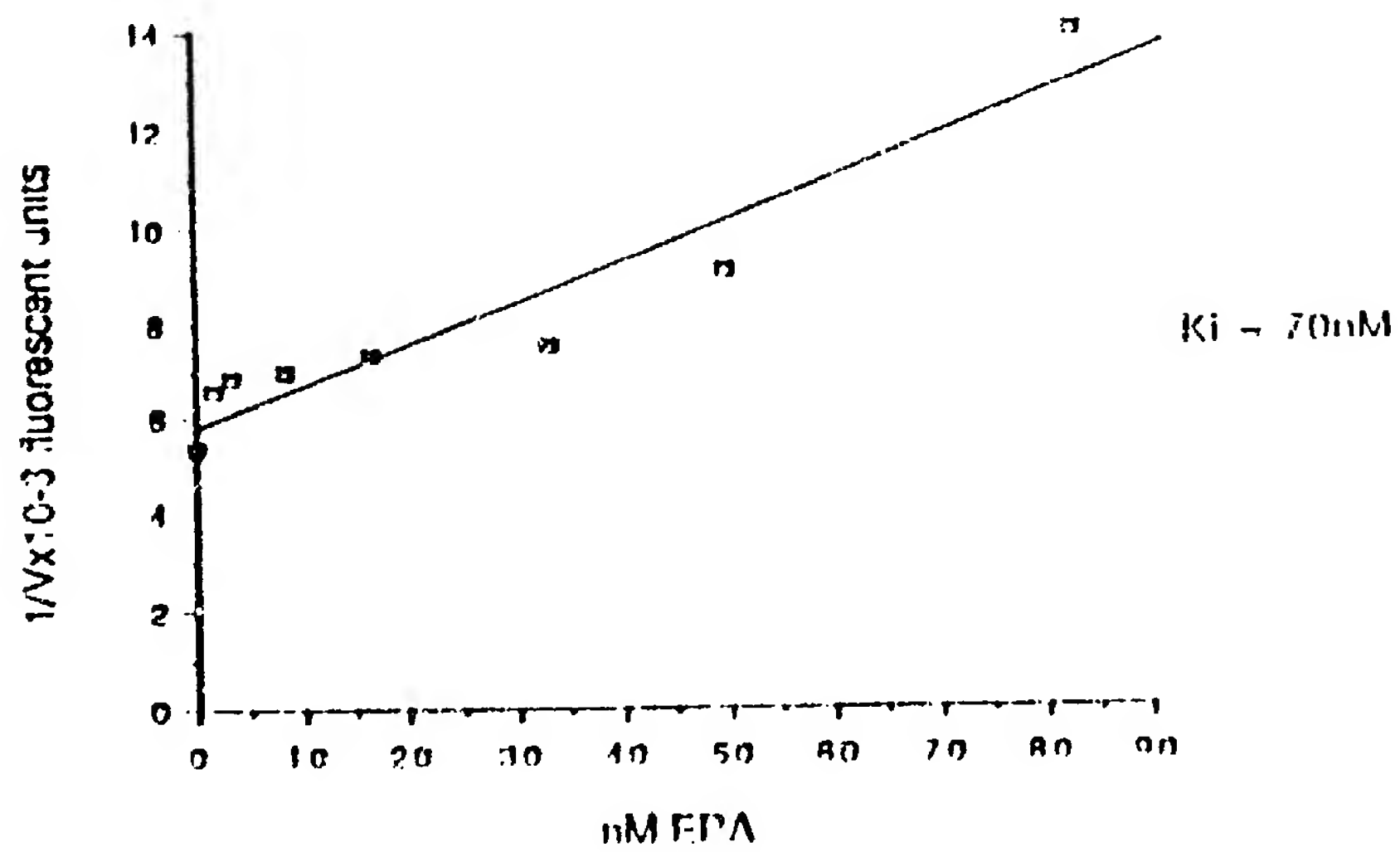
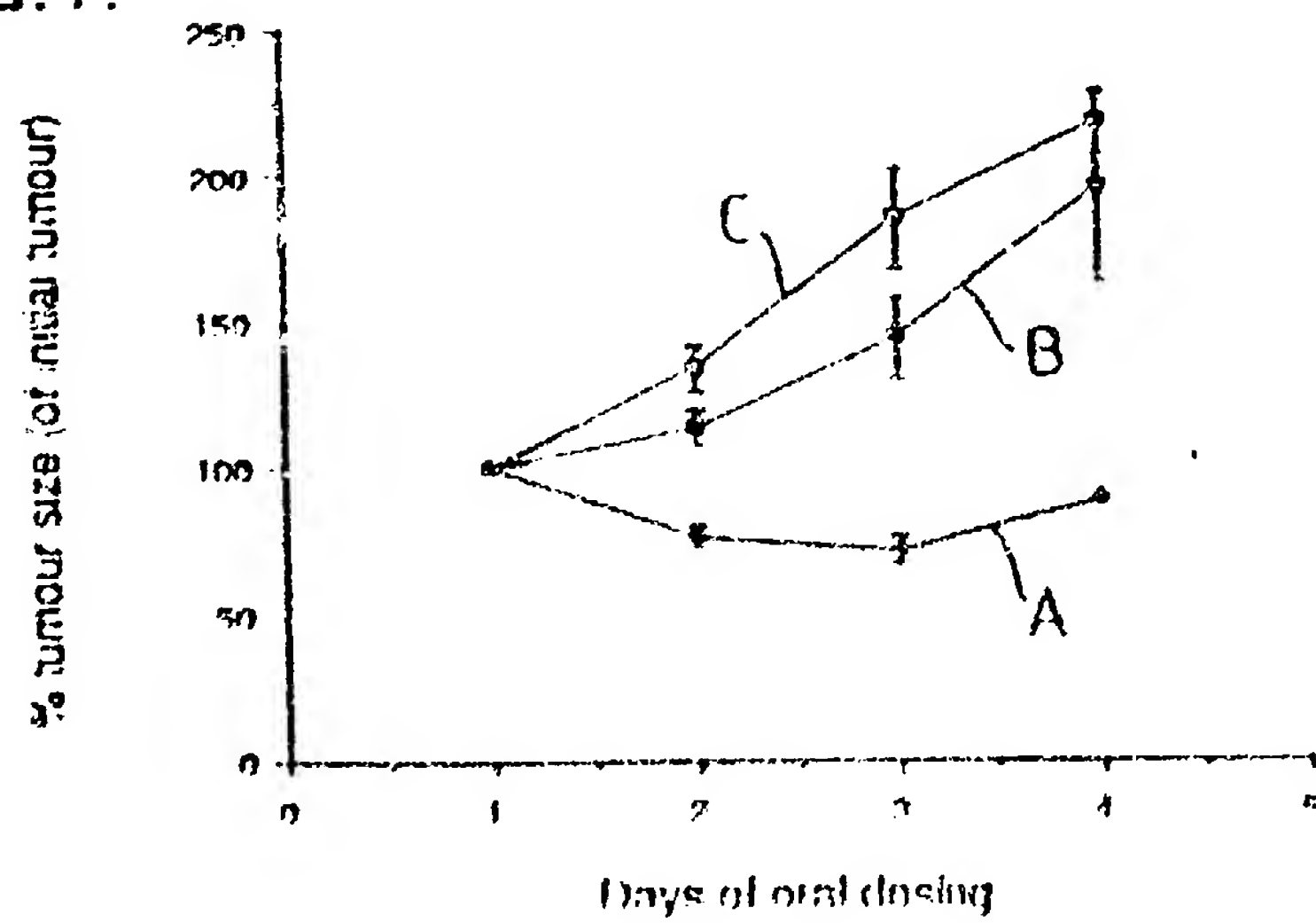


FIG. 7.

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FIG.8.

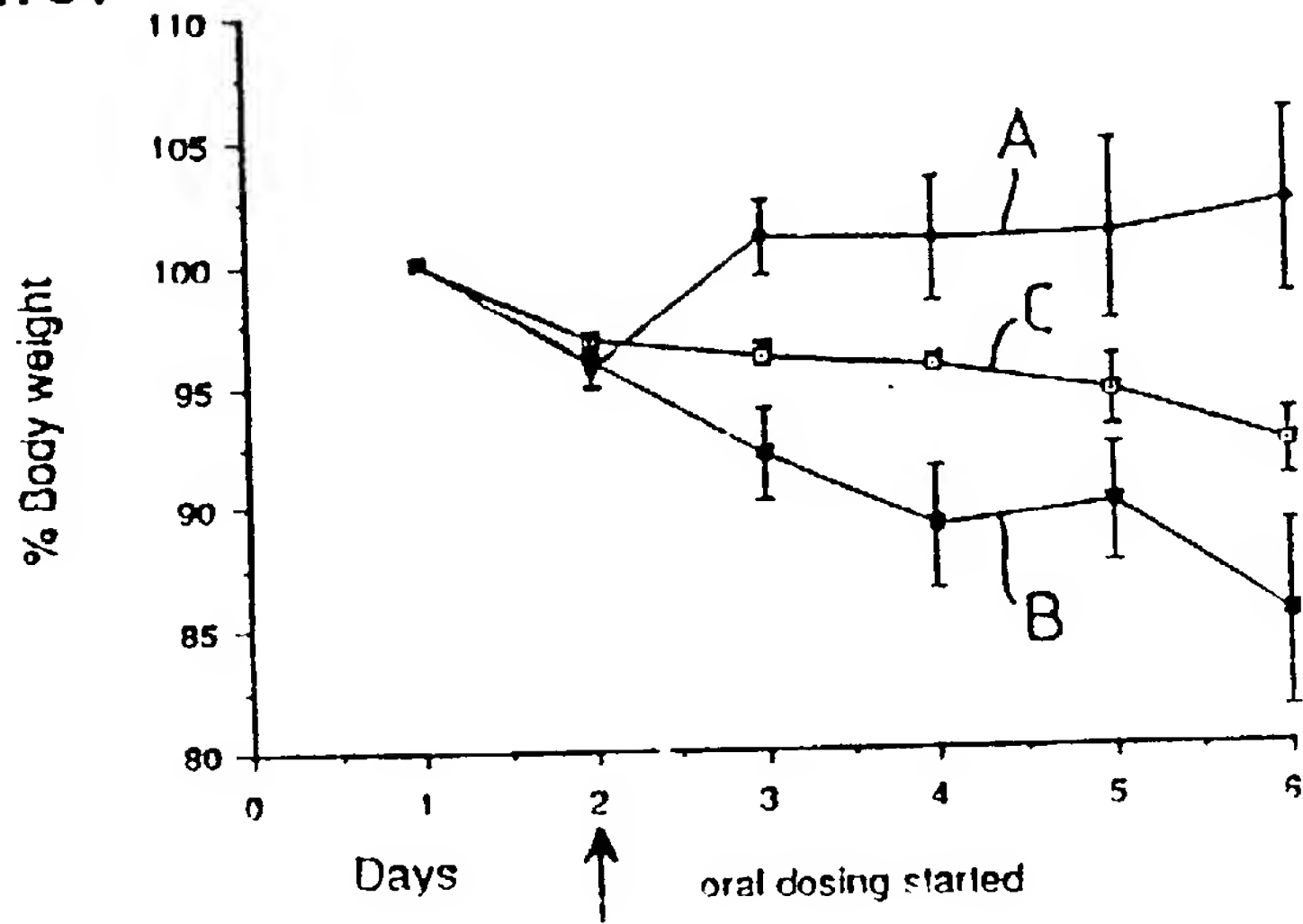
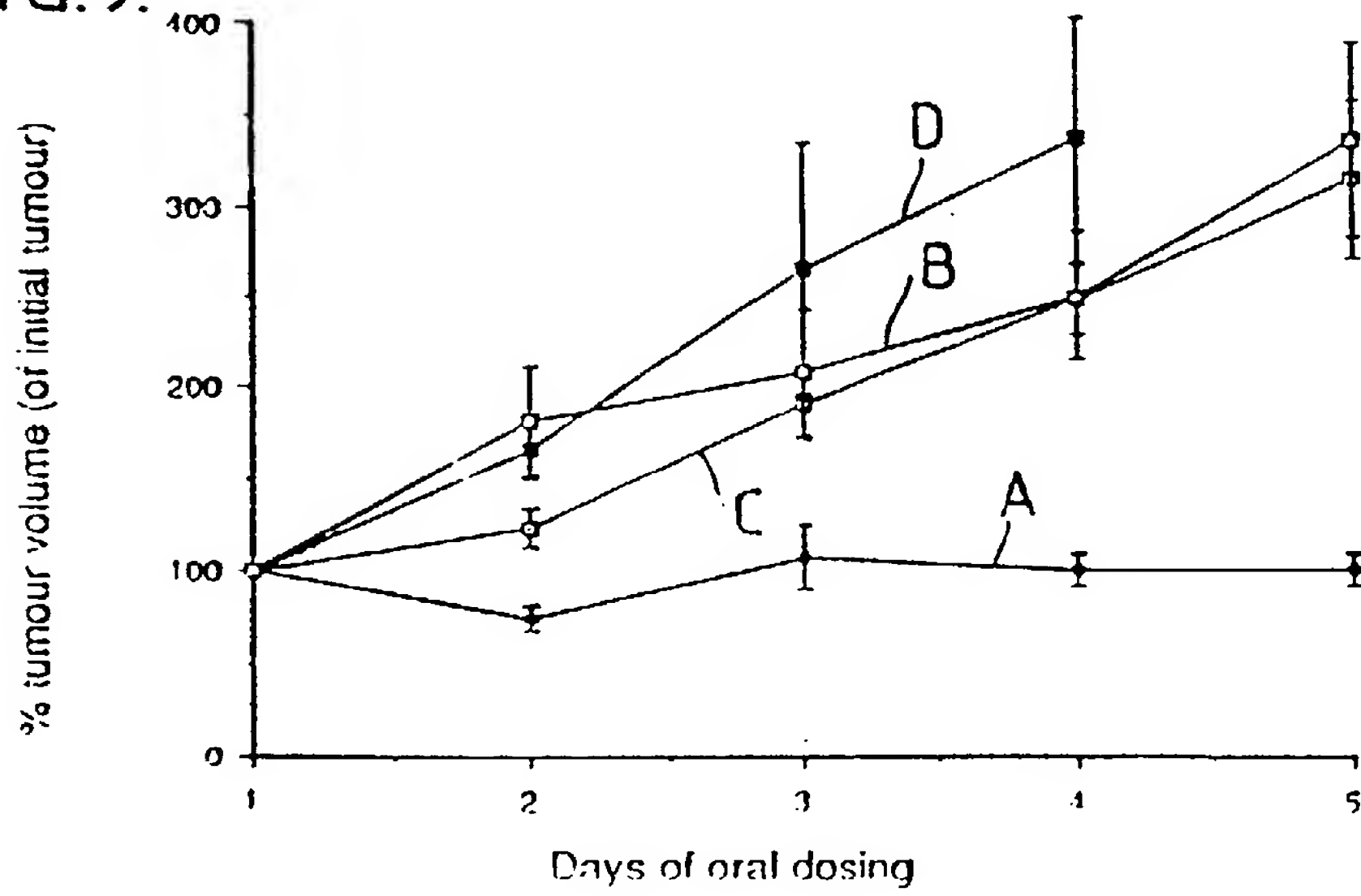


FIG.9.

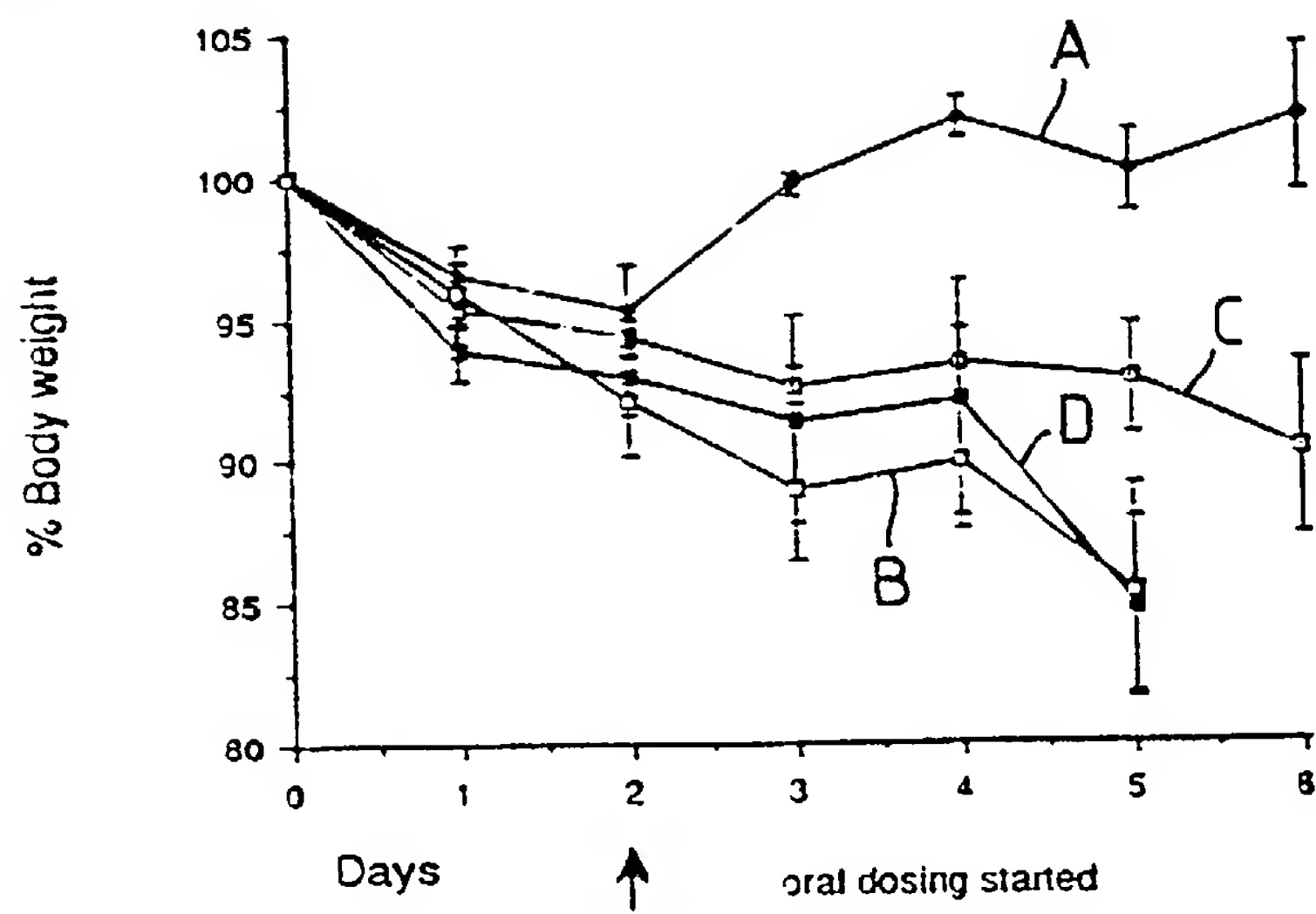


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FIG.10.



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FIG. 11A.

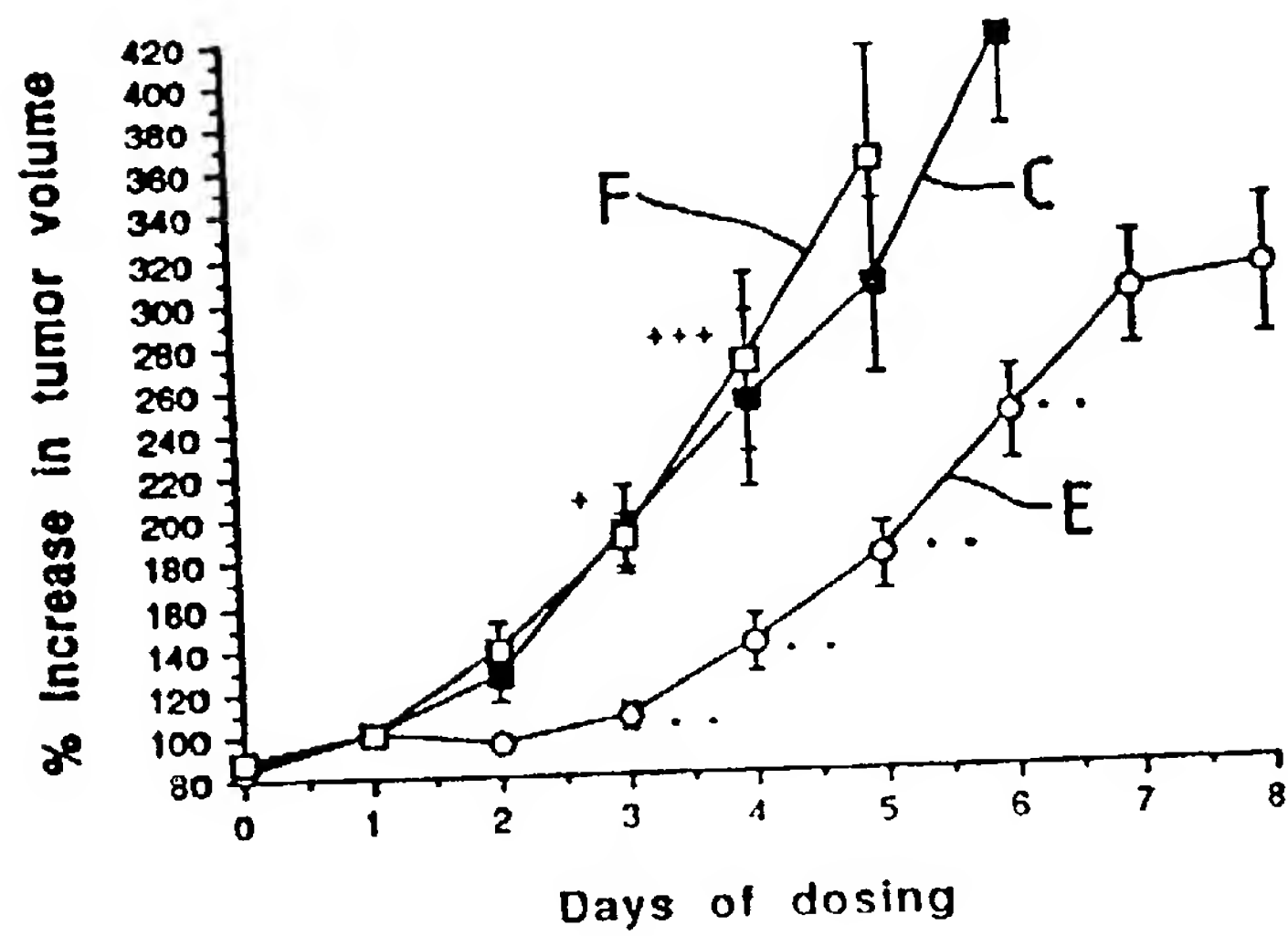


FIG. 11B.

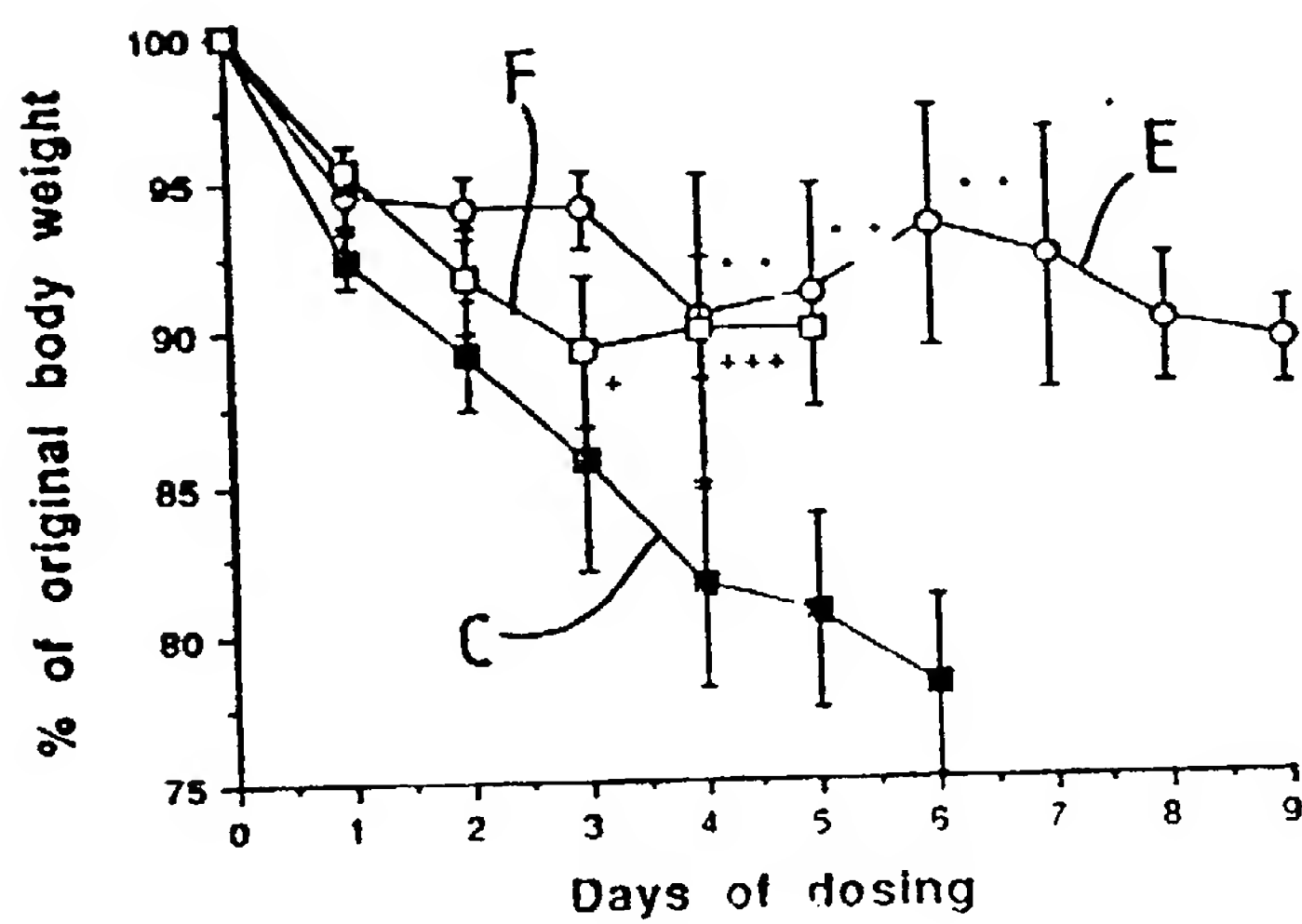
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FIG.12A.

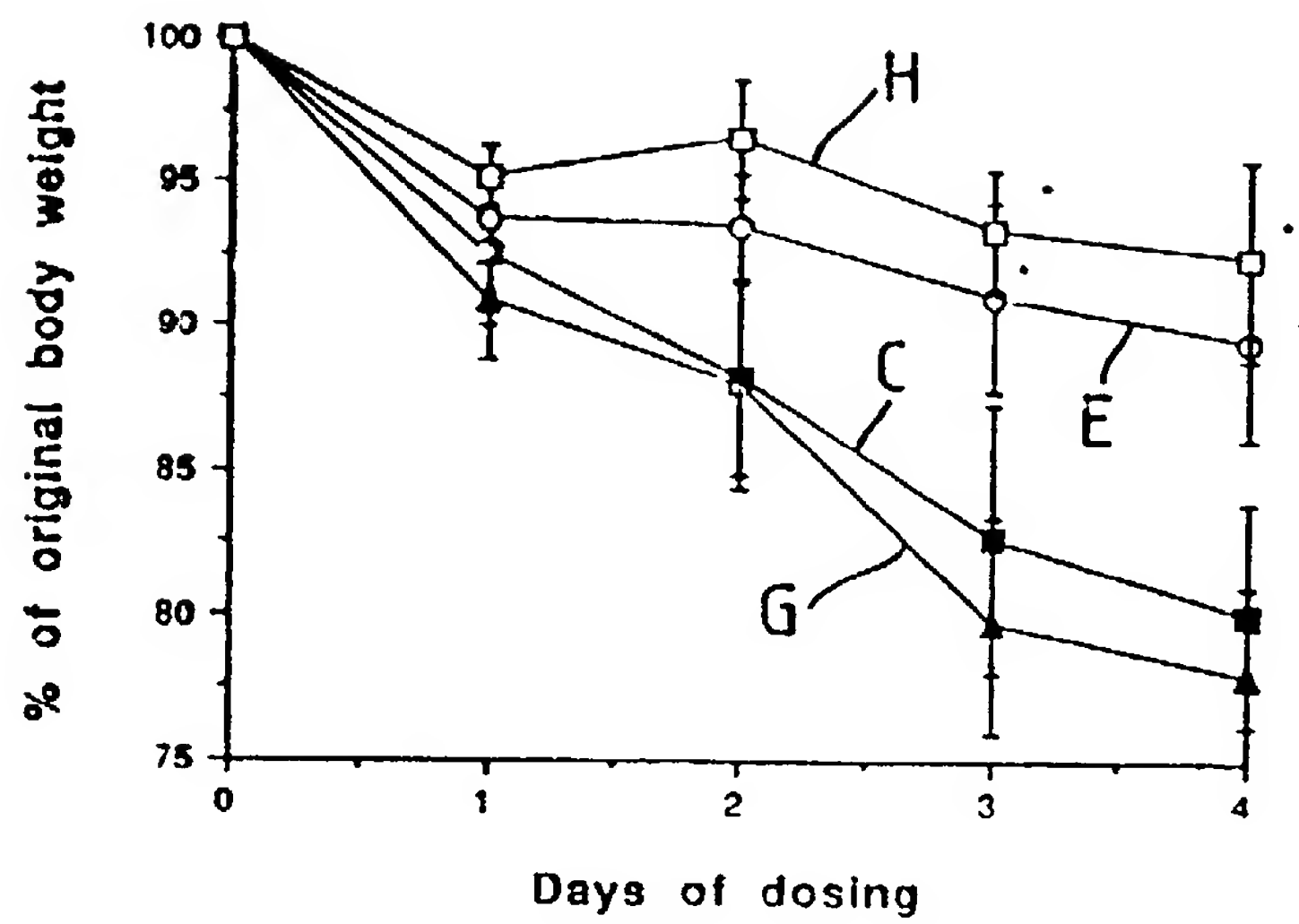
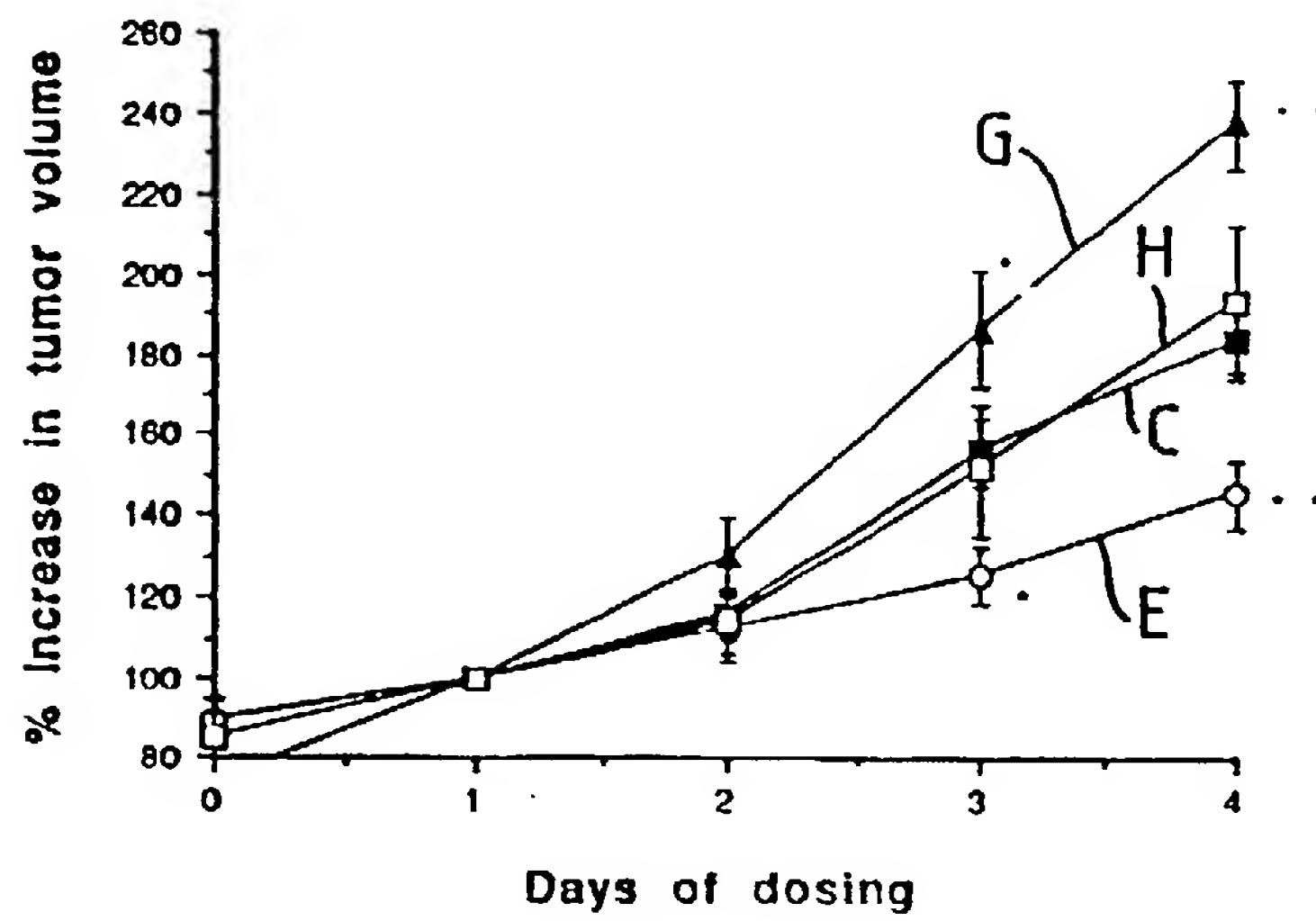


FIG.12B.

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